

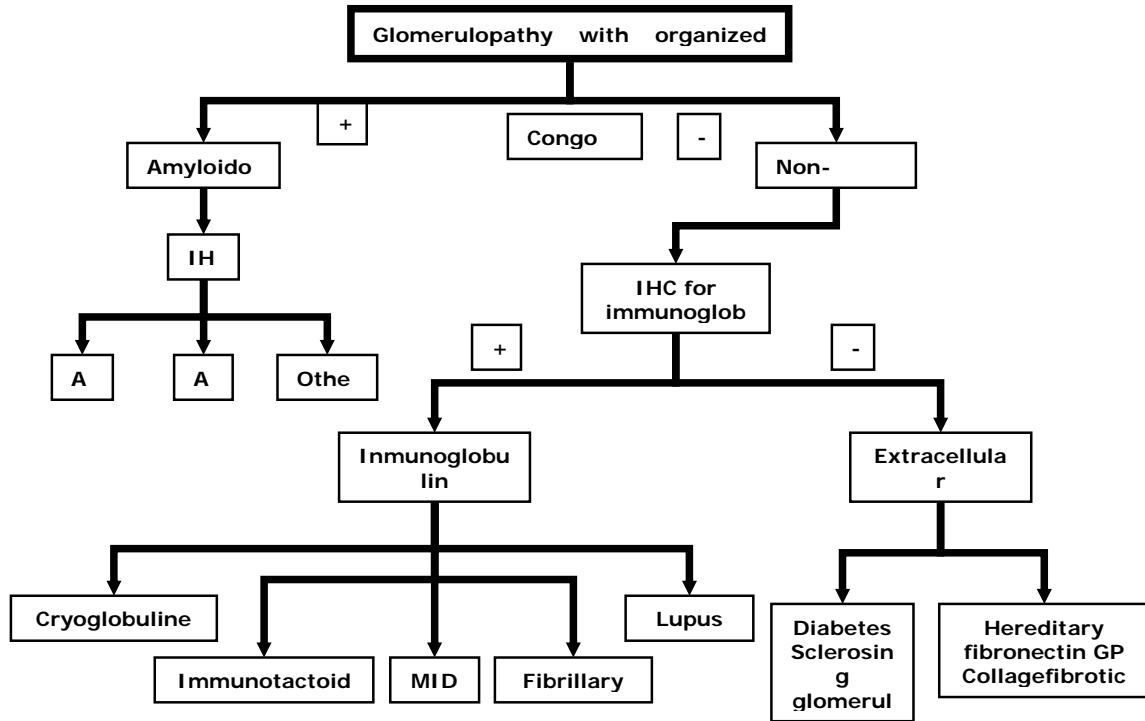
“Advances in diagnosis of paraprotein associated renal disease”

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Deposits of fibrillary structures in the extracellular matrix of the glomeruli under electron microscopy are frequent cause of renal diseases. The pathological diagnoses of these glomerulopathies with ultrastructural deposits can include either glomerular diseases, or paraproteinemic diseases, or haematopoietic diseases (Table 1) [1].

Table	1.	Glomerular	Deposition	Diseases	and	Fibrillary
Glomerulopathies						
✓	<b><u>A: Kidney (glomerular deposition diseases)</u></b>					
✓	1.	AL AH amyloidosis				
✓	2.	MIDD (non-amyloid monoclonal immunoglobulin deposition disease)				
		✓	a. LCDD (light chain deposition disease)			
		✓	b. LHCDD (light and heavy chain deposition disease)			
		✓	c. HCDD (Heavy chain deposition disease)			
✓	3.	Light chain cast nephropathy				
✓	4.	Fibrillary glomerulonephritis, Immunotactoid glomerulopathy				
✓	5.	Crystals in the podocyte, tubule, or cast				
✓	<b><u>B: Serum and/or urine (paraproteinemia/dysproteinemia)</u></b>					
✓	1.	Monoclonal gammopathy in the serum, POEMS				
✓	2.	Bence Jones protein (light chain) in the serum and urine				
✓	3.	Macroglobulinemia (monoclonal IgM)				
✓	4.	Polyclonal hyper g globulinemia				
✓	5.	Cryoglobulinemia				
✓	6.	Heavy chain disease				
✓	<b><u>C: Hematopoietic organs (Bone marrow, lymph node)</u></b>					
✓	1.	Plasmacytoma				
✓	2.	Plasma cell dyscrasia (B cell dyscrasia).				
✓	3.	Chronic lymphocytic leukemia, lymphoproliferative disorder (lymphoma)				

Evaluation of renal diseases with organized deposits requires a careful integration of information emanating from a number of sources, mainly clinical information, light microscopy conventional studies, immunohistochemistry and electron microscopy [2]. An algorithm very useful for pathologist to differentiate glomerulopathy with organized deposits was proposed by Joh [3]:



### Amyloidosis

Deposits of abnormally folded proteins with fibrillar ultrastructural appearance characterize amyloidosis. More than 25 different proteins are involved, however any protein folded by a beta structure may have amyloid characteristics [4]. A “molecular-based” classification of amyloidosis has been proposed. Eight precursor fibrils are particularly important for kidney pathology [4]:

Renal Involvement in Human Systemic Amyloidoses <sup>a</sup>		
Amyloid Protein	Precursor	Syndrome
AL/AH	Immunoglobulin; light/heavy chain	Multiple myeloma/plasma cell dyscrasia-associated, also known as primary
AA	Serum AA protein	Sporadic: secondary; familial: periodic fevers <sup>b</sup> (familial Mediterranean fever, other)
ALect 2	Leukocyte chemotactic factor 2	Renal—nephrotic syndrome and/or liver—chronic hepatitis <sup>c</sup>
Aβ <sub>2</sub> M	β <sub>2</sub> -microglobulin	Dialysis associated
ATTR	Transthyretin	Sporadic <sup>d</sup> ; hereditary <sup>e,f</sup>
AFib	Fibrinogen A α-chain	Hereditary <sup>g</sup>
AApoAI	Apolipoprotein AI	Hereditary <sup>h</sup>
AApoAII	Apolipoprotein AII	Hereditary <sup>i</sup>
ALys	Lysozyme	Hereditary <sup>j</sup>
AGel	Gelsolin	Hereditary <sup>k</sup>
ACys	Cystatin C	Hereditary <sup>l</sup>

Clinically renal amyloidosis is presented as proteinuria or nephrotic syndrome and renal failure [5]. The incidence of amyloid in patients with nephrotic syndrome or proteinuria is found in 2% to 12% of native renal biopsies [6].

Amyloid deposits cause compression on surrounding tissue, ischemia by vascular deposition and toxicity of the amyloidogenic proteins [7].

Renal involvement occurs mainly in AA and AL amyloidosis. Light chain-associated amyloidosis (AL protein) with a male predominance is the most common type in the Western hemisphere, and most of these cases are associated with lambda light chains [8]. Most cases are associated to plasma cell dyscrasias (around 70% of the cases). Usually the deposits are systemic involving multiple organs, but kidneys are the most frequently affected organs. Clinically, the presence of proteinuria, renal insufficiency, heart failure, orthostatic hypotension, peripheral neuropathy, or unexplained kidney, heart, or systemic disease are suspicious for amyloidosis. Renal amyloidosis can be defined according to the predominant distribution of amyloid deposits in renal parenchyma as a glomerular, interstitial, or vascular form of amyloidosis.

Congo red stain continues to be the gold standard for detection of amyloid deposits, with the variable morphologic phenomenon of dicroism and polarized light which shows the presence of apple-green birefringence deposits considered diagnostic of amyloid [9]. A good and precise diagnosis requires to obtain thicker sections (10  $\mu$ m).

The protein involved in amyloidosis may be determined by immunohistochemistry, using commercially available antibodies, as light chains, serum amyloid A protein, transthyretin, calcitonin, lysozyme, lactoferrin, and  $\beta_2$ -microglobulin-associated amyloidoses. Molecular diagnosis is mandatory in cases of hereditary amyloidosis and in familial Mediterranean fever (<http://fmf.igh.cnrs.fr/ISSAID/infevers/>). Electron microscopy can confirm a generic diagnosis of amyloidosis by showing the classic, randomly oriented, 8-to 12-nm diameter, nonbranching fibrils in various renal compartments [10].

Sen and Sarsik have proposed the renal amyloid prognostic score (RAPS). The sum of the amyloid scores (global glomerular sclerosis, inflammatory infiltration, interstitial fibrosis, and tubular atrophy) determines the RAPS divided into 3 grades (early, late, and advanced renal amyloidosis [5].

### **MIDD (non-amyloid monoclonal immunoglobulin deposition disease)**

Plasma cell dyscrasias may cause the deposition of light and heavy chain deposition and combination of these in the kidney [11]. For the pathological study, the lesion is similar whatever the type of chain deposited. The combination of deposition of light and heavy chain and AL amyloid has been described [12]. Joh schema of glomerular deposition disease in three groups, includes all diseases that can cause light or heavy chains in kidney (Table 1) [1].

Proteinuria and renal insufficiency are the most common manifestations. These lesions may recur in renal transplants.

Morphologically, as in amyloidosis, any compartment may be affected. The glomerular lesion is usually a nodular glomerulopathy. In MIDD, unlike amyloidosis is more frequent the kappa light chain deposition. The deposits are more intense along tubular basement membranes. In some cases, an hyperplastic vasculopathy is also found. The diagnosis may be done with immunofluorescence or immunohistochemistry. Monoclonality of the glomerular deposit is not determined

according to monoclonality in the serum or in the urine, but according to monoclonal deposition on the glomeruli by immunofluorescence study on the subtypes of IgG (IgG1–IgG4) or light chain  $\kappa$  and  $\lambda$ . Ultrastructurally electron dense punctated or powdered deposits are found [13].

### **Fibrillary glomerulonephritis**

Fibrillary glomerulonephritis was first recognized as an entity in 1977 with the presentation of a patient with nephrotic syndrome. Renal biopsy showed Congo red–negative, amyloid-like glomerular deposits [14]. Some cases are associated to neoplastic diseases, mainly lymphomas [15], or to human immunodeficiency virus–positive patients [16], but most cases are idiopathic.

The morphology in this condition is quite variable. The most frequent renal lesion is mesangial expansion by mesangial cell proliferation and mesangial deposition of eosinophilic material, however many other alterations including crescent GN have been described [17]. Usually there are deposits of IgG, C3, and light chains [14]. Ultrastructural examination identifies 15- to 25-nm diameter fibrils in the mesangium, and capillary walls. The microfibrils have no lumen, lack periodicity, and are arranged randomly. Microfibrils probably are composed of amyloid proteins, polymerized immune complexes and extracellular matrix proteins.

Some controversy still exists as to whether this condition and immunotactoid glomerulopathy are part of the spectrum of the same disease [18]. The different ultrastructure of the deposits suggests different pathogenesis but the recurrence in renal transplants suggest a common origin (Table 2) [18].

Table 2. Relationship between fibrillary and immunotactoid GP.

<b><u>FIBRILLARY</u></b>	<b><u>IMMUNOTACTOID</u></b>
Non-branching microfibrils 10-12 nm	Microtubular deposits 10–90 nm
Mesangium and glomerular basement membrane	Glomerular basement membrane, mesangium and subepithelial space
Polyclonal IgG and C3	Polyclonal IgG and C3
Membranoproliferative GN process	Membranoproliferative GN
Fifth and sixth decades of life suggests an autoimmune	The peak of occurrence is at 60 years
Subnephrotic or nephrotic range proteinuria	Subnephrotic or nephrotic range proteinuria
Primary renal disease. Relation with hepatitis C infection	Related with monoclonal gammopathy or lymphoproliferative disorders, hepatitis C virus infection, leukocytoclastic vasculitis and hypocomplementaemia
The course of FGN is usually slowly progressive. Recur in tx	The course of FGN is usually slowly progressive. Recur in tx
The presence of polyclonal IgG an autoimmune process	Probably related with type II cryoglobulinaemia

### **Immunotactoid glomerulopathy**

Schwartz et al in 1980 [19] described immunotactoid glomerulopathy. It is characterized by highly organized crystalline structures of immune deposits in the absence of systemic diseases, but frequently associated to lymphoproliferative processes and rarely with immunodeficiency virus. Clinically the patients have

proteinuria, often with associated nephrotic syndrome and hematuria. As in fibrillary GN, recurrence in transplanted kidneys has been reported [20].

Morphologically, glomeruli exhibit mesangial expansion by deposits and proliferation of mesangial cells, which may create an accentuated lobular appearance. Fluorescence studies show staining for IgG and C3 and less frequently IgA, IgM, and C1q. The diagnosis is made by ultrastructure by the presence of microtubular or cylindrical structures measuring 10 to 90 nm in diameter without periodicity or substructure [21]. The microtubules have a lumen, tend to be ordered in parallel bundles and are probably composed of immunoglobulins.

The pathogenesis of immunotactoid and fibrillary GP have not yet been elucidated fully if they are a unique or different entities [22].

### **Diabetic Fibrillosis**

Sometimes peculiar fibrils in the expanded mesangium are found in diabetic patients with nodular glomerulosclerosis [23]. Diabetic fibrillosis is only diagnosed at the ultrastructural level. Fibrils measure from 10 to 25 nm in diameter and are negative for Congo red and thioflavins T and S. Diabetic fibrillosis does not appear to have any specific clinical connotations.

### **Cryoglobulinemia**

Cryoglobulins are immunoglobulins or complexes of immunoglobulins that precipitate in the cold (usually tested at 0–4°C) and redissolve upon rewarming of serum to 37°C. Cryoglobulinemic nephropathy occurs in approximately 24% of patients with cryoglobulinemia [2]. The pathogenesis of this condition is directly related to the presence of circulating cryoglobulins [24]. Cryoglobulins cause local inflammation by deposition of immune complexes containing immunoglobulins, complement and TLR (Toll-like Receptor) [25].

The patients develop nephrotic syndrome, isolated proteinuria, hematuria, purpura, arthralgias, and other symptoms and signs of systemic vasculitis [26]. Cryoglobulinemia may be idiopathic or secondary to multiple systemic or inflammatory conditions. There are three types of cryoglobulins: type I, monoclonal, isolated, and often essential; type II, monoclonal, generally IgM with anti-polyclonal immunoglobulin activity (mostly IgG); and type III, polyclonal with more than one isotype [27]. Type III is the most common being IgG-IgM the most frequent component. Cryoglobulinemia is often associated with collagen vascular diseases, such as systemic lupus erythematosus. The prognosis is usually good.

Morphologically the glomeruli show a segmental necrotizing lesion and hyaline thrombi in capillaries [26]. The most frequent pattern of glomerular lesion is an appearance reminiscent of membranoproliferative glomerulonephritis, but mesangial proliferative GN, focal membranoproliferative GN and membranous GN have been described. Interstitial fibrosis, inflammation of the interstitium and tubular atrophy are found in less than 25% of the cases. Necrotizing vasculitis is rarely found in small sized vessels. Immunofluorescence shows subendothelial and intracapillary glomerular deposits of IgG, IgM and C3, and kappa and lambda light chains. C1q and IgA are also found in 33% and in 22% of patients, respectively [28].

Ultrastructure may show paired, curved, microtubular (cylindrical), and/or circular (annular) structures measuring 20 to 30 nm in diameter fibrillary or amorphous deposits, as well as some with fingerprints [8]. Nevertheless the diagnosis of cryoglobulinemic nephropathy requires a good clinical correlation.

### **Hereditary Glomerulopathy With Fibronectin Deposits**

This is an autosomal-dominant disease characterized by familial glomerulopathy with giant fibrillary deposits [29]. The diagnosis is made by the presence of glomerular deposits positive for fibronectin. Fibronectin GP has been seen in both sexes, with ages from 14 to 64 years. The clinical presentation is proteinuria, nephrotic syndrome, microhematuria, and hypertension. It progresses slowly to end-stage renal disease and may recur in transplant [30].

Light microscopy may suggest membranoproliferative GN or lupus nephritis, with the presence of mesangial amorphous expansion by deposits Congo red negative and fibronectin positive using immunohistochemical techniques. Immunofluorescence is nonspecific with absence or variable deposits IgG, IgM, and C3 [31]. Ultrastructurally, it is found mesangial granular or fibrillary deposits measuring 14 to 16 nm in diameter [29]. This disease may be due to mutations in FN1, located in chromosome 1q32 that contains a cluster of genes that encode regulation of complement activation [32].

### **Collagenofibrotic Glomerulopathy**

Collagenofibrotic Glomerulopathy (CG) is a rare entity characterized by deposition in the mesangial glomerulus and in the subendothelial space of type III collagen fibers [33]. The patients present with proteinuria, hematuria, hypertension and variable degrees of renal failure in adults and children. CG has occurred both occasionally and within families [34].

The glomerular lesion is characterized by mesangial hypercellularity with negative immunofluorescence. The diagnosis is based on the presence of type III collagen inside the basal membrane of the glomeruli. The fibers are in the mesangium and in the subendothelial space, but not in the subepithelial compartment or inside the basement membrane [35]. This alteration is shared with Nail-Patella Syndrome, characterized by bone and nail abnormalities, associated with proteinuria of variable degrees [36].

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